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Heme and hemolysis in innate immunity: adding insult to injury

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Heme is a vital, iron-containing prosthetic molecule present in a variety of proteins, of which hemoglobin is the most abundant. While the reactivity afforded by its central iron ion is essential for many cellular processes, it renders heme a potentially damaging molecule upon its release from hemeproteins, as it can catalyze the generation of reactive oxygen species. Severe intravascular hemolysis results in the leakage of vast amounts of hemoglobin, and subsequently, heme into the plasma. As such, heme is increasingly recognized as a major driving force for hemolysis-associated pathology including an increased risk for bacterial infections, due to its pro-oxidant, cytotoxic and immunomodulatory effects. Here, we provide a succinct review of recent, significant developments on how heme can influence innate immune functions, ranging from the maintenance of iron homeostasis by macrophages, the modulation of inflammatory responses, to its role in altering resistance mechanisms against bacterial infections.

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Introduction

Severe intravascular hemolysis can occur spontaneously due to inherited defects, which alter erythrocyte membrane stability, such as in sickle-cell disease (SCD), α or β thalassemia, and spherocytosis [1–3], or as a result of extrinsic factors such as malaria infection, severe sepsis or autoimmune hemolysis [4,5,6•]. Regardless of etiology, extensive hemolysis ultimately results in the release of vast amounts of hemoglobin into the plasma, which upon its (auto)oxidation and loss of conformational stability, readily releases its prosthetic heme groups [7]. Once released, heme is scavenged by hemopexin [8], that — upon extensive intravascular hemolysis — runs the risk of saturation, after which 'labile' heme can freely interact with other serum proteins, lipids, and exposed cells [9]. Heme consists of a protoporphyrin IX ring with a coordinated ferrous iron (Fe^{2+}) in its center, which affords heme the ability to act as a long-range electron donor/acceptor, bind diatomic gases, and importantly, act as a pseudoperoxidase via Fenton reaction [10,11]. This high reactivity of labile heme has been extensively studied and represents a well-established factor mediating tissue damage in diseases where hemolysis occurs [6•,12,13].

It has long been recognized that hemolytic disorders predispose to potentially deadly bacterial infections [14–17], exemplified by the high rate of invasive bacterial infections in children with SCD [18], or studies showing disproportionately high bacteremia rates in malaria patients [17]. This complication has often been attributed to hemolysis-derived increased iron availability for bacterial growth [19,20]. However, recent data indicate that labile heme (i.e. heme, which has been released from hemeproteins and can freely interact with serum components and cells), can directly influence host immune responses, implicating an important role for labile heme in hemolysis-driven immunosuppression. This review aims at providing a concise update on recent, significant discoveries on the interplay between hemolysis, labile heme accumulation and innate immune effector mechanisms.

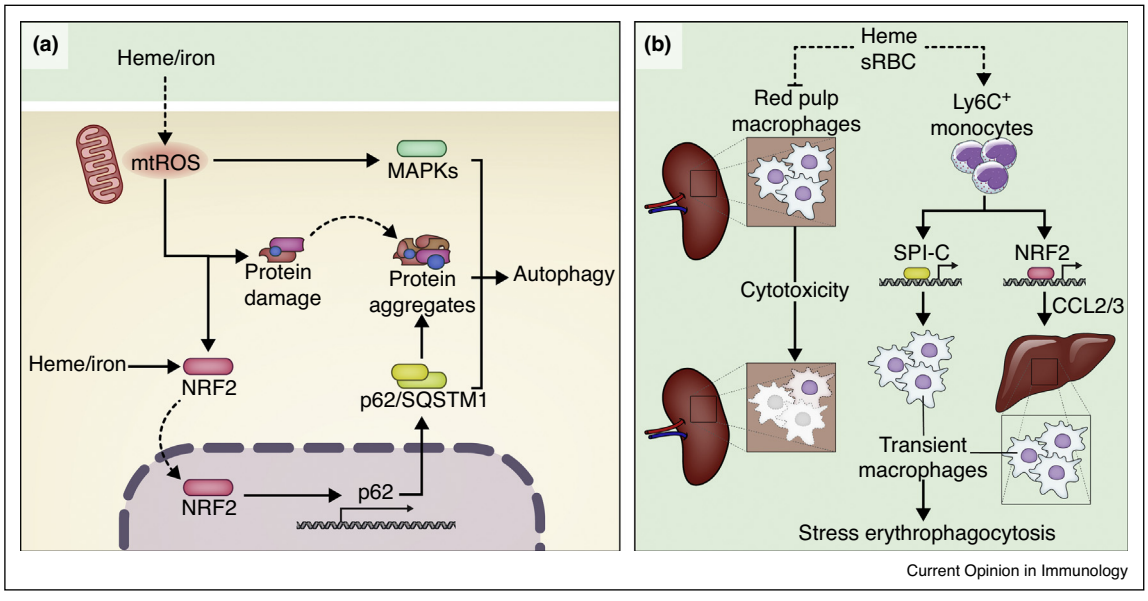
Mechanisms to offset macrophage heme *overdose*

Tissue-resident macrophages are central orchestrators of both immune responses and tissue homeostasis. Besides sensing/eliminating pathogens, and coordinating responses to noxious stimuli, macrophages are crucial for maintaining iron homeostasis at steady state [21], as well as in response to infection or tissue damage [22]. Moreover, iron homeostasis itself can heavily influence inflammation and immune responses [23]. Under homeostatic conditions, splenic red pulp and bone-marrow macrophages (RPM and BMM, respectively) are especially equipped to catabolize high heme levels that result from the uptake of senescent erythrocytes [21]. However, increased erythrocyte damage or intravascular hemolysis can lead to acute heme accumulation in macrophages, and subsequent heme-triggered cell death [24]. Macrophages have been shown to undergo programmed necrosis upon

heme treatment via the generation of reactive oxygen species (ROS) and autocrine tumor necrosis factor (TNF) signaling [24]. Although precise molecular mechanisms are lacking, there is a large body of evidence showing that ROS generation by heme is a major force in driving heme-mediated cytotoxicity, both for hematopoietic and non-hematopoietic cells [24–26]. To counter the deleterious effects of heme, macrophages promptly upregulate a number of protective mechanisms, which are induced by heme itself [27]. Classically, these protective responses involve heme catabolism by heme oxygenase 1 (HO-1), iron sequestering by ferritin (FTH) and the generation of bilirubin, a potent lipophilic antioxidant [27,28]. This is further supported by a concurrent antioxidant stress response controlled by nuclear factor (erythroid-derived 2)-like 2 (NRF2) [29]. Interestingly, NRF2 has recently been shown to be necessary for the formation of distinct p62/Sequestosome 1 protein aggregates in macrophages upon heme exposure [30] (Figure 1a). Specifically, both heme and iron released from heme triggered mitochondrial ROS (mtROS) generation, p62 upregulation and mitogen-activated protein kinase (MAPKs) signaling, thereby inducing autophagy [30], possibly to cope with the stress incurred by increased protein synthesis and ROS-mediated oxidative protein damage [31] (Figure 1a). However, since protein aggregates can be toxic *per se* [32], it remains unclear whether this mechanism in fact protects, or facilitates heme-triggered cytotoxicity.

The loss of erythrophagocytic macrophages following severe hemolysis could precipitate an inexorable buildup of damaged erythrocytes, labile heme accumulation in the plasma, and organ damage. Recently, two distinct, yet surprisingly similar mechanisms have shown that intravascular hemolysis induces the transdifferentiation of circulatory monocytes into fully functional iron-recycling macrophages, effectively compensating for RPM and BMM loss [33••,34••] (Figure 1b). One study directly implicated labile heme in the transdifferentiation of CD11b^{hi}, Ly6C^{+} monocytes into RPM and BMM both *in vitro* and *in vivo* following phenylhydrazine-(PHZ) triggered hemolysis [33••]. This is achieved via heme-triggered polyubiquitination and proteasomal degradation of the transcriptional repressor BTB domain and CNC homolog 1 (BACH1) [35], allowing the expression of SPI-C, a transcription factor required for RPM/BMM differentiation [36] (Figure 1b). Similarly, stressed erythrocyte delivery *in vivo* or PHZ-triggered hemolysis were shown to specifically induce Ly6C^{hi} monocyte migration into the liver in a CCL2- and CCL3-dependent manner, where they differentiate into transient iron-recycling macrophages [34••] (Figure 1b). Remarkably, although the authors did not assess a direct contribution of labile heme, this transdifferentiation program critically depended on NRF2, a transcription factor whose activity is strongly induced by heme and iron [29] (Figure 1b), suggesting that labile heme or iron might be at least partially required for Ly6C^{hi} monocyte

Figure 1



Macrophage mechanisms to offset heme toxicity. **(a)** Heme induces autophagy via the generation of mtROS, and subsequent NRF2 and MAPK activation. This induces p62/sequestosome 1 (SQSTM1) protein aggregation. **(b)** Heme triggers transdifferentiation of Ly6C^{+} monocytes into erythrophagocytic transient macrophages to compensate for loss of red pulp macrophages. Solid lines indicate direct links, dashed lines indicate indirect links, or a node transition.